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PRINCIPAL INVESTIGATOR: Xiang-Dong Fu, PhD

CONTRACTING ORGANIZATION:
University of California, San Diego
La Jolla, CA 92093-0621

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6. AUTHOR(S)
Xiang-Dong Fu, PhD
Betty Diamond

E-Mail: xdfu@ucsd.edu

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14. ABSTRACT
We have been awarded a project designed to use a molecular signature strategy to develop new therapeutics against metastatic breast cancer. As originally proposed, we have been pursuing the projects in three phases: (1) Define the molecular signature tightly linked to the gene expression program underlined the Epithelial-to-Mesenchymal Transition (EMT), (2) Conduct chemical screening by following the EMT program by using a novel pathway-centric technology we recently developed, and (3) characterize leading components identified from the screen on cell and animal models to evaluate their effects in inhibit breast cancer metastasis. The goals set for the first year are to define genes regulated by Twist, Slug, and Sip1 in a triple negative breast cancer cell line. We have accomplished all set goals. In addition, we have proceeded to design and test oligo pool to be used for chemical screening and adapt our assays in the high throughput format on our robots. We are well positioned to move to the next phase, which is to further refine the assay conditions and then conduct the proposed chemical screening.

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Progress Report

Grant Number: **BC101584**

Principal Investigator: **Xiang-Dong Fu**

Grantee Institution: **University of California, San Diego**

Project Title: **Chemical Strategy to Translate Genetic/Epigenetic Mechanisms to Breast Cancer Therapeutics**

Project Period: **7/1/2011 – 6/30/2013**

Progress Period: **7/1/2011 – 6/30/2012**

Introduction

This project is designed to use a molecular signature strategy to develop new therapeutics against metastatic breast cancer. We have recently published this novel pathway-centric technology developed on a prostate cancer model (Li et al., 2012b). The goal fro this project is to define a panel of genes that are tightly associated with breast cancer transition from initial epithelial morphology to mesenchymal-like cells. This process, known as the EMT transition, has been shown to play a critical role for breast cancer metastasis. Once the panel of EMT-linked is defined on a triple negative breast cancer, the next step is to conduct small molecular screens against such collection of responsive genes. Resulting candidate hits will be characterized on cell and animal models to determine their biological effects in inhibiting breast cancer metastasis to distal organs. This approach is uniquely suited to attack disease pathways in the absence of druggable targets that can be pursued by conventional approaches.

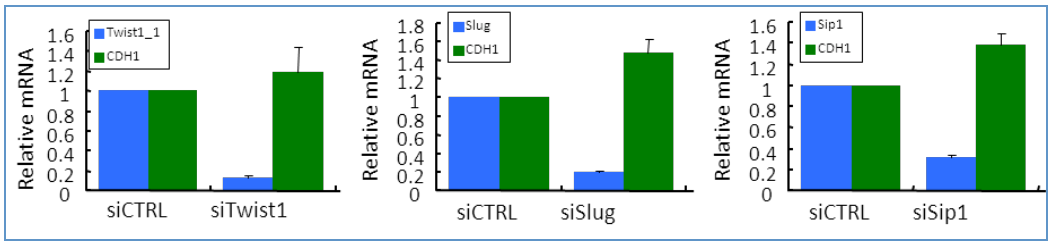
Body of the Progress Report

According to our original Statement of Work, the project is to proceed in three phases.

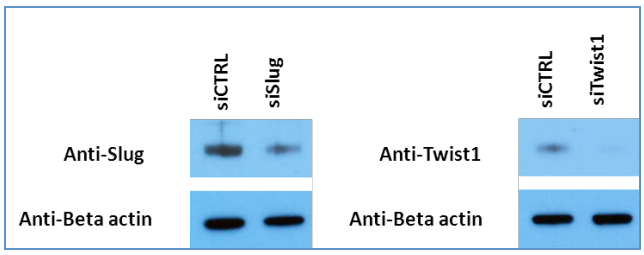
<u>Aim 1: Define the gene signature associated with the EMT program</u>

(1) Perform RNAi on SUM1315 cells against Twist, Snail, Slug, and SIP1
(2) Confirm the RNAi efficiency by Western blotting
(3) Conduct RNA-seq analysis on both wt and RNAi knockdown cells
(4) Conduct bioinformatics analysis of the RNA-seq data to define regulated genes in each case
(5) Deduce specific and common gene signature trigger by individual EMT inducers
(6) Validate a subset of regulated genes by real time PCR

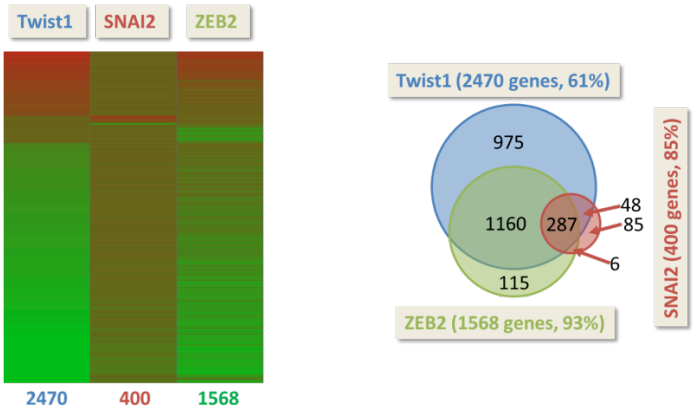
We have carried out RNAi against Twist1, Slug (SNAI2) and Sip1 (ZEB2) on SUM1315 cells (a cell line characteristic of triple negative breast cancer cells). As shown in the figure, the knocking-down efficiency determined by RT-qPCR is high in all three cases, while control RNAi showed little effect. The RNAi effects appear quite specific, as there were no measurable effects on a non-target gene (CDH1).



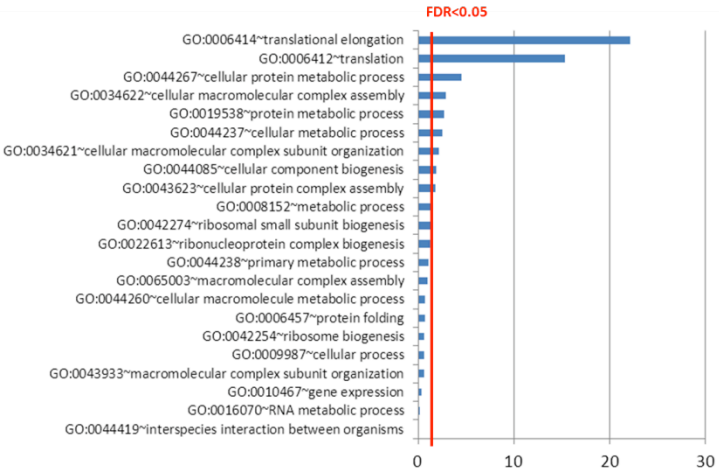
We confirmed efficient down-regulation of targeted Slug and Twist by Western blotting. As shown in the figure below, we achieved significant reduction of both proteins. The effect is especially striking on Twist. We have not yet done Western blotting with anti-Sip1 because of the poor quality of the antibody in our hands. We are in the process of testing other antibodies from various resources.



The success of these initial experiments allowed us to initiate genome-wide analysis of genes regulated by these transcription factors. We have developed a robust RNA-seq strategy by determining the digital information on the 3' end of each transcript expressed from the human genome (Fox-Walsh et al., 2011). We have applied this technology to detect genes responsive to knockdown of Twist, Snail, and ZEB2. Heat map of altered gene expression is shown below on the left.



As expected, knockdown of each gene induced a unique spectrum of responses; however, there is a significant overlap among responsive genes to all three genes involved in the EMT transition (see the Venn diagram above on the right). By GO term analysis, these common 287 genes are enriched in translation and cellular metabolic processes, as shown below.



We have validated some of the responsive genes by RT-qPCR and are continuing this effort to ensure the robustness of the data before proceeding to the next phase. In conclusion, we have accomplished all set goals for the first year. As described below, we have already initiated some experiments set for the second year.

Aim 2: Screen for small molecules that can specifically repress the EMT program

(1) Design and test oligo pairs targeting the defined gene signature
(2) Test cell culture conditions for chemical screening
(3) Treat the cell with the collection of 4,500 human experienced compounds library
(4) Identify lead compounds and confirm by independent methods
(5) Determine EC50 for a handful drug leads selected for follow-up studies
(6) If resources permit, initiate the screen against the larger 100,000 compounds library

Based on the RNA-seq results, we have selected 205 genes (202 common genes + Twist1, Sip1 and Slug) to design RASL-seq oligo pairs (Li et al., 2012a). We also include 26 house-keeping genes in this oligo pool as internal specificity controls. As described in the proposal, this is an evolving process. After initial design and synthesis, we need to test them experimentally to (1) eliminate those that give rise to too many counts (this will consume our sequence space), (2) compare the results with our genome-wide and RT-qPCR results to ensure that individual oligo pairs report the anticipated effects in regulated gene expression, (3) re-design and re-synthesize those that are not efficient in targeting intended genes, and (4) conduct initial screen on our robot to determine if the cell culture system and the oligo pool can be adapted to high throughput operation. We have been following these exact steps in refining each step in preparation for the proposed chemical screening.

Thus far, we have validated a set of oligos based on their detected outcomes that match the RNA-seq and RT-qPCR results. We are now in the process of re-designing and re-testing many of them in order to produce a highly robust pool of oligos for chemical screening.

In parallel, we have initiated the efforts in adapting the cell culture system for high throughput operation. We have shipped the cell line to Dr. Sheng Ding's lab at Gladstone/UCSD for them to test culture in the 384-well format, determine the growth/treatment conditions to ensure optimal cell density for drug treatment, and carry out some test run on their screening robot.

In conclusion, we have initiated some of the experiments intended for the first half of the second year. We are still in the process. If things go well, we will start chemical screening near the end of the summer or at the beginning of the fall.

Aim 3: Test the effect of drug leads on cell and animal models

(1) Conduct Western blotting of epithelial and mesenchymal markers on SUM1315, Bt594, and MDA157 cells before and after treatment with drug leads
(2) Perform cell migration and invasion assay on mock-treated and drug-treated cells
(3) Test the effect of leading candidates on breast cancer metastasis on nude mice

As described in the original proposal, these tasks will be pursued once we have candidate compounds. We thus have not performed any of the described experiments in the first year.

Key Research Accomplishments

- Conducted RNAi on a breast cancer model
- Performed genome-wide analysis to define the EMT-linked molecular signature
- Designed, synthesized, and tested initial oligo pool for chemical screening
- Tested the screening procedure on our assay robot
- Adapted the cell model on screening robot
- Tested and optimized conditions for chemical screening

Reportable Outcomes

We have published three papers on gene signature and chemical screening strategies this year, although these works were supported by other resources prior to funding of the current project. In addition, we have been presenting our technology development efforts in a recent Society for Laboratory Automation and Screening (SLAS) meeting in San Diego.

Conclusion

We believe that we are progressing exactly as we described in our proposal. We encountered some problems in validating some of initially synthesized oligos, which might be related to pool quality of chemical synthesis. We are now addressing these problems. Other than this technical issue, we are on target to our set goals.

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